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Amplification of the Xq28 FRAXE Repeats: Extreme Phenotype Variability?

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We report on a new case of FRAXE mutation identified through the screening of a population of FRAXA-negative mentally retarded individuals. The index case, a 4-year-old boy with distinct minor anomalies and mental retardation with severe verbal impairment, his older brother, referred to as normal, and the mother have undergone careful clinical and molecular evaluation. The molecular defect, characterized by standard Southern blot analysis, is represented by a hypermethylated "full mutation" in the 2 boys and by a unique, altered, presumably unmethylated, band in the mother, which is interpreted as a "premutation." The cytogenetic analysis failed to detect a folate-sensitive Xq27-28 fragile site in either "fully mutated" individual. The phenotype and intellectual performance of the 15-year-old brother of the propositus appeared completely normal. Our propositus shares some traits with previously described FRAXE-mutated subjects, suggesting an association with the Xq28 molecular defect; nevertheless, we find it difficult to reconcile the molecular identity and phenotypic difference in these mutated members of the same family. This could be a case of extreme phenotypic variability or a result of a more complicated molecular mechanism. © 1996 Wiley-Liss, Inc.

KEY WORDS: FRAXE, triplet repeats, phenotype

INTRODUCTION

Since the new tools for the direct molecular diagnosis of the fragile X syndrome (FRAXA) have become available, we have started a screening program to identify FRAXA-mutated subjects in a population of individuals affected by mental retardation of unknown cause. All of the FRAXA-negative individuals are subsequently reevaluated to exclude the possibility of association between their mental impairment and the instability of a recently discovered region of trinucleotide repeats, located about 600 Kb distal to the FMR-1 repeats, designated as the FRAXE region [Flynn et al., 1993]. The FRAXE mutation belongs to the category of dynamic mutations [Richards et al., 1992] and seems to behave very similarly to FRAXA, where the mutational mechanism is represented by an inherited pathologic expansion of the unstable CGG triplet repeats located at the 5′ end of the FMR-1 gene and by methylation of the adjacent CpG island [Bell et al., 1991; Pieretti et al., 1991; Verkerk et al., 1991; Vincent et al., 1991; Sutcliffe et al., 1992]. The FRAXE repeat region, which is polymorphic in the normal population, with a number of GCC triplets variable between 6 and 25, has been found to expand to more than 200 copies in mentally retarded males expressing an Xq28 folate-sensitive fragile site [Knight et al., 1993]. The mechanism by which a large expansion of the repeats in Xq28 may be responsible for a clinical phenotype could be the association with hypermethylation of the region, documented in all the affected FRAXE subjects, as it is in FRAXA fully mutated individuals [Hamel et al., 1994; Knight et al., 1994]. This hypermethylation would inactivate important regulatory sequences of a yet undiscovered gene. Mild mental retardation in males seems to be the prevalent aspect of the FRAXE phenotype [Mulley et al., 1995], although a definite correlation between the molecular defect and a clinical syndrome is still under discussion, due to the small number of cases observed to date and to the variability of the phenotypes described. We have identified 1 FRAXE-mutated individual among 180 subjects tested. We report the clinical and molecular characterization of the index case, his mother, and his mutated, phenotypically normal brother.

CLINICAL EVALUATION

The propositus was referred to us because of mental retardation of unknown cause with severe speech impairment. His intelligence, evaluated at the age of $3\frac{1}{2}$ years with a Stanford-Binet test, gave an IQ of 68 and

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a mental age of 28 months. The psychometric profile at different items was not homogeneous, showing a particular deficit in verbal-skill items. His actual verbal skills are limited to few monosyllables, and he always prefers mimicking to verbal production. Language comprehension, although largely insufficient for his age, is more satisfactory than production, allowing him to have very simple relationships with peers. The boy also has midface hypoplasia, hypotelorism and epicanthic folds, micrognathia, abnormal dental morphology (Fig. 1), and clinodactyly of the 5th fingers. These traits are not found in either parent or in the 15-year-old brother, whose psychometric evaluation evidenced a homogeneous profile and an IQ of 92 on a Wechler intelligence scale. This brother also had FRAXE mutation.

MOLECULAR AND CYTOGENETIC CHARACTERIZATION

The study has been conducted with conventional Southern blot of genomic DNA digested with the restriction enzyme HindIII and hybridized with the probe OxE20. This probe–enzyme combination, which detects a 5.2-Kb band in normal individuals, allowed us to identify the mutation of our proband as a broad band of larger size (Fig. 2). The mutation, if properly resolved, shows a certain degree of somatic heterogeneity, Δ = 900–1,600 bp (Fig. 3). Double digestions of the patient's DNA with HindIII and the rare cutters EagI and BssHII were performed. The HindIII restriction fragment remained substantially unaltered, documenting the condition of hypermethylation of a CpG island in the region (Figs. 2, 3). The mother and the 15-year-old brother have been tested with HindIII and BamHI digestions to confirm the presence of a mutation of the FRAXE region and to exclude the possibility of a rare HindIII polymorphism (BamHI data not shown). Both carry a mutation at the FRAXE locus: the mother has a

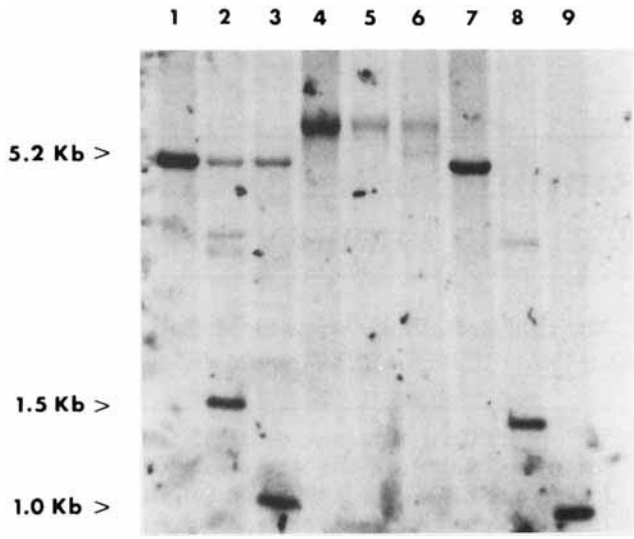

Fig. 2. Southern blot analysis of genomic DNA. **Lanes 1–3:** Normal female control. **Lanes 4–6:** Propositus. **Lanes 7–9:** Normal male control. Digestion by HindIII in lanes 1, 4, 7; HindIII/BssHII in lanes 2, 5, 8; and HindIII/EagI in lanes 3, 6, 9. The arrows indicate the normal 5.2-Kb HindIII fragment and the 1.5- and 1-Kb fragments generated by complete digestion of the active X chromosome with the rare cutters BssHII and EagI, respectively.

Fig. 1. The propositus at 4 years old. Note the midface hypoplasia, hypotelorism, epicanthic folds, and micrognathia.

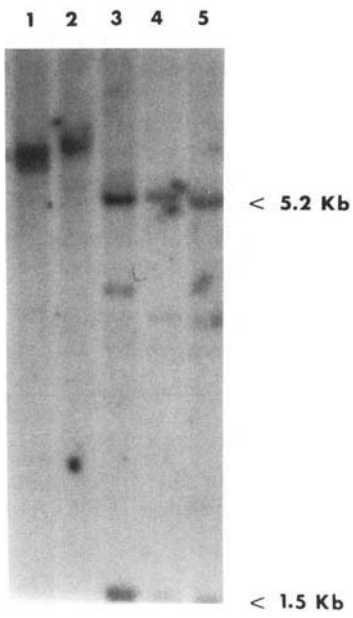

Fig. 3. Southern blot analysis of genomic DNA double digested by HindIII/BssHII. **Lane 1:** Brother. **Lane 2:** Propositus. **Lane 3:** Mother. **Lanes 4–5:** Normal female controls. The arrows indicate the normal 5.2-Kb HindIII band and the 1.5-Kb fragment generated by complete BssHII digestion of the active X chromosome.

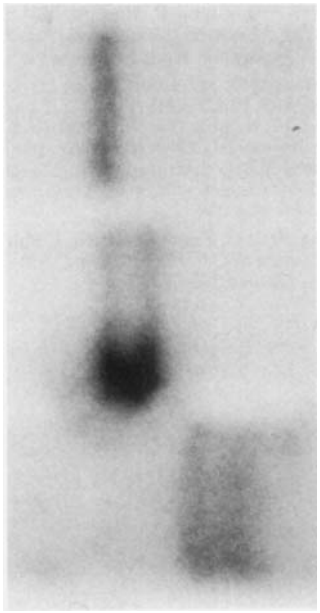

Fig. 4. Southern blot analysis of genomic DNA digested by HindIII. **Left lane:** Normal control. **Middle lane:** Mother. **Right lane:** Brother.

mutated allele with a Δ of approximately 200 bp, and the brother shows 2 main bands of expansion, with a Δ of about 600 and 1,200 bp, respectively (Fig. 4). Assessment of the methylation status evidenced a situation of complete hypermethylation for the brother but was not conclusive for the mother, whose pattern of double digestion resembles that of a normal female (Fig. 3). Peripheral blood samples from the 2 "fully mutated" boys were cultured in TC199 folate-deficient medium supplemented with 10% fetal calf serum (FCS) for 96 hr. The patient's peripheral blood was also cultured in TC199 10% FCS incubated with MTX for 24 hr. At least 150 metaphases for each subject were scored by QFQ banding. The cytogenetic analysis failed to detect folate-sensitive Xq27-28 fragile sites in either individual; the analysis of the patient's karyotype by high-resolution techniques was also normal.

DISCUSSION

The FRAXE family we describe is, at the molecular level, a typical example of transmission of a dynamic mutation with trinucleotide sequence CGG/GCC. We think the mother carries a "premutation," based on the size of the detected alteration and on the results of the methylation analysis. The unexpectedly normal banding pattern generated by the double digestion with the methylation-sensitive enzymes might be due to the presence of EagI and BssHII, previously unrecognized restriction sites located between the probe and the repeats, preventing the identification of expansions of these sequences in the absence of methylation. The molecular analysis of the 2 boys shows an identical situation of classic "full mutation" characterized by a dramatic expansion of the inherited mutated allele and complete hypermethylation of the whole region. Despite the molecular identity, compatible with the inactivation of the putative FRAXE gene and the phenotypic expression of the disease, the sibs present a very different phenotype. The phenotype of the propositus is very different from that of FRAXA individuals, and several traits, such as mental retardation with particular verbal impairment and midfacial hypoplasia, overlap with the phenotype of other reported FRAXE patients [Hamel et al., 1994; Knight et al., 1993, 1994; Mulley et al., 1995]. His brother, carefully assessed, is clinically completely normal.

This case seems to represent additional evidence of the relation between a particular phenotype and the FRAXE mutation. Nevertheless, it raises questions about the inconsistent expression of this mutation and the molecular or biological bases of it, questions that have an important impact for molecular diagnosis and genetic counseling. The hypothesis of a somatic heterogeneous distribution of the molecular defect with specific damage of a target tissue might explain the extreme phenotypic variability observed, but it needs to be confirmed by the study of the function of one or more genes that would be inactivated by the FRAXE full mutation. In the meantime, the identification of premutated or fully mutated individuals necessarily implies the analysis of every member of a FRAXE family at risk of being a carrier, even when there is a completely negative phenotype, and the uncertainty about genotype–phenotype correlation greatly limits the actual possibility of offering prenatal diagnosis to FRAXE families. Finally, the fact that both of our fully mutated subjects are cytogenetically negative may be interpreted as an indication of the need to rely only on molecular analysis for the diagnosis of FRAXE, as has been proposed for FRAXA.

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